7/16 - IGEM

SO- Pick colonies from proteins A(D1 and D2) and B(D3) and streaked out on new LB Kan 50 plates. Incubated overnight at 37°C

SO- PCR reaction to amplify H1 out of Labrenzia cp4 ~~had~~ have not been able to amplify out of mixed (MCW) community

H is heterostaggered, because it contains an internal cut site

PCR reaction:

H1	H2	Neg 1	Neg 2
µL Q5 2x master mix	25µL Q5 2x mastermix	25µL Q5 2x mastermix	25µL Q5 2x master
25µM HFwd (#71)	1µL 25µM HFwd (#71)		1µL 25µM HFwd (#71)
25µM HRevI (#72)	1µL 25µM HRevA (#73)		1µL 25µM HRevI (#72)
µL NF H2O	23µL NF H2O	25µL NF H2O	23µL NF H2O
Labrenzia cPlolony	1 Labrenzia cPlolony	1 Labrenzia cPlolony	1 Labrenzia cp4 colony

Thermocycler Protocol:

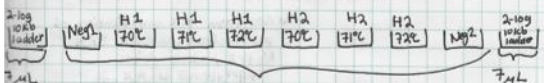
Step	Temp	Time
Initial Denaturation	98°C	3 minutes
35 cycles	98°C	10 seconds
	70°C-72°C*	30 seconds
	72°C	45 seconds
Final Extension	72°C	2 minutes
Hold	10°C	∞

Primers contained N's, so ~~their~~ annealing temperatures cannot be determined (may range from 70°C to 72°C). ~~H1 and H2~~ 3 replicates of H1 and H2 were made, so each temp could be tested

Moved to -20°C when done

8/18/16 - IGEM

:00 - Ran 1% Agarose gel of H's PCR products at 110V for 40min.



nalyzing F's transformations

Antibiotic Resistance	Volume Plate	~# of colonies	notes
Kan	10 µL	12	4 small, 8 large
Kan	100 µL	~100	1/4 small
Kan	400 µL	~1000	1/4 small
chloro	10 µL	field	cannot select ~~good~~ isolated colonies
chloro	100 µL	↓	↓
chloro	400 µL		

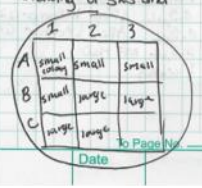
Will continue on with the 10 µL ~~chloro~~ Kan plate.

Pick 8 colonies (4 small and 4 large) and streak onto one ~~grid~~ square of a 3x3 grid on a LB Kan 50 plate. Put rest of colony left on plate into a PCR tube for colony PCR. Placed plate in 37°C incubator.

Per PCR tube	
12.5 µL	2x Green Mix
0.5 µL	FFwd1 (Primer # 68)
0.5 µL	FRev (Primer # 70)
11.5 µL	NF H_2O
1 colony	

Thermocycler
1) 95°C for 3:00
2) 95°C for 0:30
3) 67°C for 0:30
4) 72°C for 3:00
5) 2-4 x34 times
6) 72°C for 5:00
7) 10°C ∞

Drawing of 3x3 Grid



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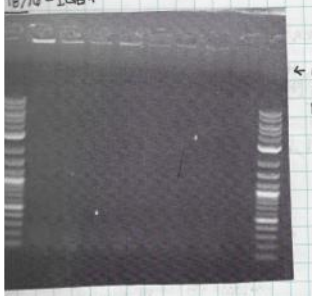

← Gel from PCR amplification of PBR H from Laborenzia CP4
No PCR products visible
↳ 4th attempt at PCR w/o success → will analyze primers again

00 - PCR check of Gene A (D1 and B D2) and B (D3)
Picked ½ of a colony w/ P10 pipette tip from plates streaked on 08/17/16 and used for colony PCR (outlined below)
Picked second half of colony w/ wooden stick and used grew up in 3mL LB Kan for 4-6 in 37°C shaker

Colony PCR:
ran a negative control of each without a colony

Gene A
12.5 µL 2x Green Mix
0.5 µL A Fwd 1 (Primer G2)
0.5 µL A Rev (Primer G4)
11.5 µL NF H_2O
1 colony

Gene B
12.5 µL 2x Green Mix
0.5 µL B Fwd (Primer G5)
0.5 µL B Rev (Primer G6)
11.5 µL NF H_2O
1 colony

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08/18/16 – IGEM

Thermocycler for Genes A and B PCRs

STEP	TEMP	TIME
Initial Denaturation	95°C	3:00
35 cycles	95°C	0:30
	66°C	0:30
	72°C	1:00
Final Extension	72°C	2:00
Hold	10°C	∞

Ran 1% Agarose Gel at 110V for 20min of A and B's PCR and F's PC screen

-A | | AD1 | 2-log ladder | AD2 | BD3 | ladder | -B | | |

FA1 | FA2 | FA3 | ladder | FB1 | FB2 | water | FB3 | FC1 | FC2

10 μL 7 μL 10 μL 7 μL 10 μL

More likely that something ~~wasn't~~ went wrong with the PCR runs than all 3 colonies did not have the insertions

15:30 – Split each liquid culture (AD1, AD
into 3 15 mL tubes
3 mL LB
3 μL Kan (50 mg/mL)
30 μL culture
grow up overnight in 37°C shaker

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8/16 - IGEM

- freezer stocks (800 µL cells, 250 µL 50% glycerol) and dist
prepped remaining cells overnight growths of AD1, AD2, BD3
eluted w/ 50 µL Buffer EB

eezer Stock #	Insert	Plasmid	Label
89	A	Kan	AD1
90	A	Kan	AD2
91	B	Kan	BD3

odropped

Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/2
A D1	SS Gabrielle Tender	8/19/2016 11:43:07 AM	328.1	ng/µl	6.561	3.460	1.90	2.29
A D2	SS Gabrielle Tender	8/19/2016 11:44:01 AM	285.5	ng/µl	5.709	3.007	1.90	2.29
B D3	SS Gabrielle Tender	8/19/2016 11:44:48 AM	516.2	ng/µl	10.324	5.462	1.89	2.24
MCS-2::Undecaheme D1	SS Gabrielle Tender	8/19/2016 11:45:31 AM	94.3	ng/µl	1.886	0.971	1.94	2.17
MCS-2::Undecaheme D2	SS Gabrielle Tender	8/19/2016 11:46:09 AM	82.6	ng/µl	1.653	0.854	1.93	2.18
EB	SS Gabrielle Tender	8/19/2016 11:46:44 AM	-0.3	ng/µl	-0.006	-0.005	1.14	0.35

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08/22/16 -IGEM!

Streaked FB3 and FC1 for singles on a LB Kan50 plate
Incubated at 37°C overnight

08/23/16 -IGEM!

Picked ½ of a colony from F plates (one from FB3 and one from FC1 w/ a p10 pipette tip, colony PCR (outlined below) to verify insert.
Picked second ½ of colony w/ wooden stick and grew up in 3mL of LB Kan 50 in 37°C shaker for 4-6 hrs

PCR
12.5μL 2x Green Mix
0.5μL FFwd Primer (#68)
0.5μL FRev Primer (#70)
11.5μL NFH2O
1 colony

3 PCR tubes
- neg (no colony)
- FB3 (FB3 colony)
- FC1 (FC1 colony)

"master mix"
37.5μL 2x Gre
1.5μL FFwd
1.5μL FRev
34.5μL NF Ha
25μL per tub

Thermocycler

1) 95°C for 3:00
2) 95°C for 0:30
3) 67°C for 0:30
4) 72°C for 3:00
5) 2~4 x 34 times
6) 72°C for 5:00
7) 10°C for ∞

Digestion for tomorrow's ligation (small scale to verify correct digestion)
Plan is to dig digest B w/ EcoRI and SpeI, F w/ XbaI and PstI, and A w/ EcoRI and
↳ ligate together B and F into plasmid from A (or find linearized backbone)

AD1	AD2	BD3	
0.5μL EcoRI	0.5μL EcoRI	0.5μL EcoRI	37°C for 1 hr
0.5μL PstI	0.5μL PstI	0.5μL SpeI	
1μL AD1	1μL AD2	0.5μL BD3	
1μL 10x cutsmart	1μL 10x cutsmart	1μL 10x cutsmart	
7μL NFH2O	7μL NFH2O	7.5μL NFH2O	

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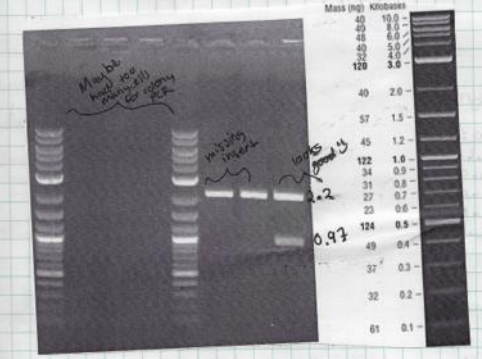
08/23/16 - IGEM!

Ran 1% agarose gel of PCR and RE digests at 110 V for 40min

10kb	FB3	FB3	FC1	10kb	AD1	AD2	BD3	10kb	
7 µL	PCR rxn 5 µL			7 µL	RE digest 10 µL + 2 µL 6x dye			7 µL	



Split FB3 and FC1 liquid cultures into 3 liquid cultures each

3mL LB

3µL Kan 50mg/mL

30µL previous liquid culture

incubate in 37°C shaker overnight

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24/16 - iGEM

de freezer stocks (800 µL cells, 250 µL 50% glycerol)

ezer stocks #	DNA
92	iGEM F-B3
93	iGEM F-C1

niprepped DNA (eluted w/ 50 µL Buffer EB) and nanodropped

mple ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
EM FB3	SS Gabrielle Tender	8/24/2016 12:23:08 PM	166.4	ng/µl	3.327	1.752	1.90	2.28
EM FC1	SS Gabrielle Tender	8/24/2016 12:24:03 PM	247.9	ng/µl	4.957	2.613	1.90	2.19

striction Enzyme Digest

D3	FB3	FC1	Kan vector (2 µL BD3)	Chloro vector
. BD3	6 µL FB3	4 µL FC1	~~5 µL FB3~~ ~~5 µL pSB1K3~~	5 µL pSB1C3
Cutsmart Buffer	3 µL cutsmart	5 µL cutsmart	5 µL cutsmart	5 µL cutsmart
EcoRI	1 µL XbaI	1 µL XbaI	1 µL EcoRI	1 µL EcoRI
SpeI	1 µL PstI	1 µL PstI	1 µL PstI	1 µL PstI
L NFH2O	38 µL NFH2O	40 µL NFH2O	84 µL NFH2O 42	38 µL NFH2O

°C for 1 hr

ed to separate backbone from insert

an 1% Agarose Gel of all but chloro backbone (doesn't contain insert)
t 110V for 40 min

BD3	2kb 10kb	FB3	FC1	2kb 10kb	Kan vector
0 µL	2 µL	50 µL		20 µL	50 µL
0 µL 6x dye		+10 µL 6x dye			+10 µL 6x dye

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08/24/16 - iGEM

PCR cleaned up Chloro vector (eluted w/ 50 µL Buffer EB)

Gel purified BBD3 and Kan vector (eluted w/ 50 µL Buffer EB)

No insert in either F vector both
Lpicked 5 colonies from each FB3 and FC1 plates
Grew up in 3 mL LB Kan 50

08/25/16 - iGEM

Made freezer stocks of all 10 F growths (FB3A, FB3B, ..., FC1D, FC1E) (800 µl cells, 2[illegible] SG

Labeled each one 9%, but then put a label w/ tape on side

Miniprepped DNA (eluted w/ 25 µL Buffer EB)
Laccidentally labeled FC1C as FC1D, so can't differentiate - relabeled the two "FC1Ds" as FC1C/D and FC1D/C

Nanodropped

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280
	psB1K3 digested and purified	SS Gabrielle Tender	8/25/2016 10:43:37 AM	10.5	ng/µl	0.210	0.092	2.29
	Buffer EB	SS Gabrielle Tender	8/25/2016 10:44:25 AM	0.4	ng/µl	0.008	-0.010	-0.79
	psB1K3 digested and purified	SS Gabrielle Tender	8/25/2016 10:45:16 AM	27.6	ng/µl	0.551	0.285	1.93
	iGEM BD3 digested and purified	SS Gabrielle Tender	8/25/2016 10:46:13 AM	5.7	ng/µl	0.113	0.035	3.21
	psB1C3 digested and purified	SS Gabrielle Tender	8/25/2016 10:47:05 AM	3.6	ng/µl	0.072	0.009	8.04
	iGEM FB3 A	SS Gabrielle Tender	8/25/2016 10:48:03 AM	152.5	ng/µl	3.050	1.573	1.94
	iGEM FB3 B	SS Gabrielle Tender	8/25/2016 10:48:51 AM	149.7	ng/µl	2.994	1.550	1.93
	iGEM FB3 C	SS Gabrielle Tender	8/25/2016 10:49:42 AM	151.3	ng/µl	3.027	1.566	1.93
	iGEM FB3 D	SS Gabrielle Tender	8/25/2016 10:50:28 AM	125.9	ng/µl	2.519	1.286	1.96
0	iGEM FB3 E	SS Gabrielle Tender	8/25/2016 10:51:29 AM	129.7	ng/µl	2.594	1.340	1.94
1	iGEM FC1 A	SS Gabrielle Tender	8/25/2016 10:52:22 AM	107.3	ng/µl	2.145	1.096	1.96
2	iGEM FC1 B	SS Gabrielle Tender	8/25/2016 10:53:22 AM	115.5	ng/µl	2.310	1.186	1.95
3	iGEM FC1 C/D	SS Gabrielle Tender	8/25/2016 10:54:20 AM	122.5	ng/µl	2.450	1.267	1.93
4	iGEM FC1 D/C	SS Gabrielle Tender	8/25/2016 10:57:57 AM	103.1	ng/µl	2.061	1.037	1.99
5	iGEM FC1 E	SS Gabrielle Tender	8/25/2016 10:58:54 AM	119.6	ng/µl	2.393	1.222	1.96
6	Buffer EB	SS Gabrielle Tender	8/25/2016 10:59:47 AM	0.5	ng/µl	0.011	-0.005	-2.29

do not look good will repeat

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8/25/16 -iGEM!

~~Restriction~~
Digested each F vector w/ XbaI and PstI (~~did not~~
only did 10 µL digestion (didnot want to waste enzyme or Buffer)

Per tube
1 µL DNA
0.5 µL XbaI
0.5 µL PstI
1 µL CutSmart Buffer
7.5 µL NFH2O

37° for 1hr

"Master Mix"
5 µL XbaI
5 µL PstI
10 µL cutsmart
75 µL NFH2O

9 µL per tube

Found Digested and undigested plasmid backbones..
Nanodrop & and image to determine length, purity and conc.

Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280
GEM clean cut plasmid K 7/12/16	SS Gabrielle Tender	8/25/2016 12:04:34 PM	15.1	ng/µl	0.303	0.176	1.72
digested plasmid C	SS Gabrielle Tender	8/25/2016 12:05:45 PM	26.2	ng/µl	0.523	0.305	1.72
iGEM K plasmid 7/11/16 KC	SS Gabrielle Tender	8/25/2016 12:06:48 PM	288.1	ng/µl	5.762	3.033	1.90

Ran 1% Agarose Gel at 110V for 20min

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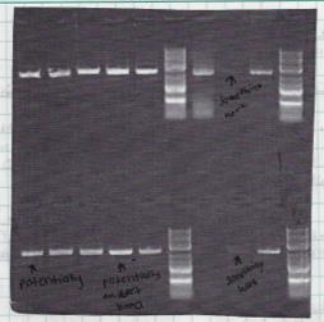
Date: 08/23/16 - iGEM



Sol'n: amplify F out of MCL extract, FC1 D, FC1 A. amplify B out of BD3.

F_MCL
2 µL 3E8 extract
1 µL 25 µM FFwd1
1 µL 25 µM BRev
25 µL 2X
21 µL NF H20

F_FC1D
12.5 µL 2X
0.5 µL FC1DX
0.5 µL template
1 µL FFwd1
1 µL FRev1
25 µL 2X
22.5 µL NF H20

F_FC1A
0.5 µL FC1A
1 µL FFwd1

B_BD3

Made 1:10 dilutions of FC1D, FC1A, BD3 (use these in PCR)
~~Made 1:4 dilutions of BFwd primer #5 and BRev primer #66~~
~~B master mix~~

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PCR from pg 71

F mastermix

1

~~0.75~~ µL 100 µM FFwd1 (primer #68)

~~0.75~~ µL 100 µM FRev (Primer #70)

~~2.75~~ µL Q5 2x mastermix

~~4.50~~ µL NF H_2O

B master mix

1 µL 100µM BFwd (primer # 65)

1 µL 100 µM BRev (primer # 66)

6 µL NF H_2O

F_MCL

1 µL MCL SE extract

49 µL F mastermix

F_ FCID

1 µL 1:10 FCID D/C

49 µL F mastermix

F_ FC1A

1 µL 1:10 FC1A

49 µL F mastermix

B_BD3

1 µL 1:10 BD3

2 µL B mastermix

2.5 µL Q5 2x master

22 µL NF H_2O

F_neg

1 µL NF H_2O

49 µL F mastermix

Thermocycler:

STEP	TEMP	TIME
Initial Denaturation	98°C	30sec
35 cycles	98°C	10sec
	72°C	30 sec
	72°C	1 min
Final Extension	72°C	2min
Hold	10°C	∞

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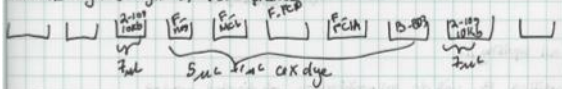
08/26/18e - iGEM

I do not trust any of the PCRed or digested Kan vector (psB1K3.m1) left for me. I will instead digest it out of psB1K3.m1::BD3

~~digestion~~

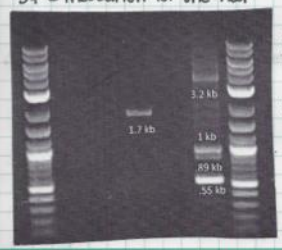
Ran 1% agarose gel of PCR products



→ Digestion of psB1K3.m1::BD3 to recover psB1K3.m1
Did 4 50 µL reactions
Per tube (did 4x master mix and split it into 4 tubes)
7 µL EcoRI-HF
1 µL PstI
2 µL iGEM BD3
5 µL 10X cutsmart Buffer
42 µL NF H20

4x master mix
9 µL EcoRI
4 µL PstI
8 µL iGEM BD3
20 µL 10X cutsmart
164 µL NF H2O

37°C incubation for one hour



I am very confused, because it amplified out of F. FCID but not MCL ~~gDNA~~ genomic DNA

PCR clean up F_FCID
Labeled "~~F-FCID~~" "iGEM F"
Gel purified B-BD3 by cutting out 1kb band and purifying (look on next page for more info)

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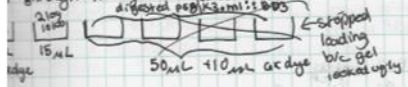
8/26/16 - iGEM

purified pSB1K3+m1 and gene B
through 1% agarose gel for 40 min
digested pSB1K3+m1 : BD3



was all squishy...

amplified B, will do gel purification of digested product

2x50 µL rxns:

~~Master Mix~~

per tube:	2ml:
1 µL EcoRI	2 µL EcoRI
1 µL SpeI	1 µL SpeI
1 µL iGEM BD3	2 µL iGEM BD3
5 µL 2x cutsmart	5 µL 2x cutsmart
42 µL NF H2O	42 µL NF H2O

C for 1 hr

cleaned up F from MCL and F from FC1A too (eluted w/Shut Buffer EB cartr)

Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
F PCRed from FC1D/C	SS Gabrielle Tender	8/26/2016 2:35:21 PM	8.6	ng/µl	0.173	0.083	2.08	2.68
F PCRed from MCL	SS Gabrielle Tender	8/26/2016 2:36:21 PM	6.4	ng/µl	0.128	0.053	2.42	2.26
F PCRed from FC1A	SS Gabrielle Tender	8/26/2016 2:37:06 PM	6.5	ng/µl	0.130	0.055	2.38	2.04
er EB	SS Gabrielle Tender	8/26/2016 2:37:53 PM	-1.0	ng/µl	-0.021	-0.015	1.37	0.35

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